SUGAR-CONTAINING AMPHIPHILIC OLIGOMERS

ROCO   OCOR

SUGAR

R—OCO   OCOR

$\parallel$ O

OCHN

C—Enkephalin

O

Hydrolysis cleaves the
lipophilic moieties and
generates a hydrophilic
sugar-drug conjugate.

Brain peptidases cleave
the sugar moiety to
generate a free peptide.

FIGURE 1A

ROCO   OCOR

SUGAR

ROCO   OCOR

—NCCH₂OCH₂CH₂OCH₂CHN

C—Enkephalin

FIGURE 1B

ROCO   OCOR

SUGAR

ROCO   OCOR

—OCNCH₂CH₂OCH₂CH₂OCHN
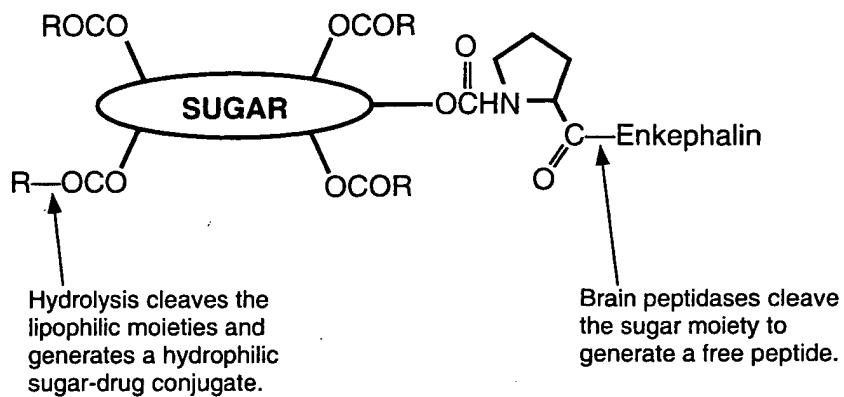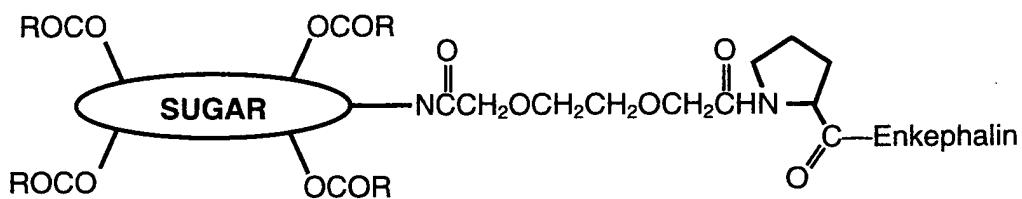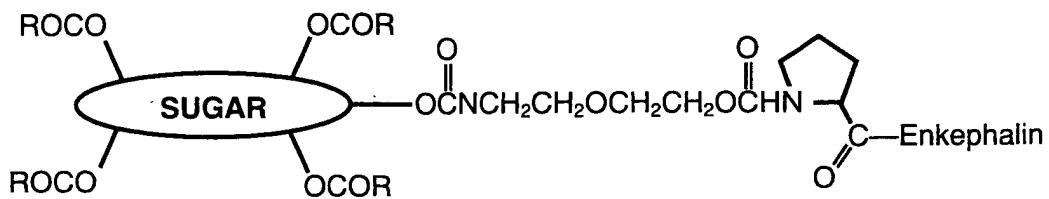
C—Enkephalin

FIGURE 1C

Stability of Enkephalin and Cetyl-PEG$_2$-Enkephalin in Rat Brain Homogenate

2 % Rat Brain in

PBS buffer, pH 7.4, 37 C

Peptide = 60 ug/mL

Percent of Initial Concentration

Time (min)

Met-Enkephalin-Lys
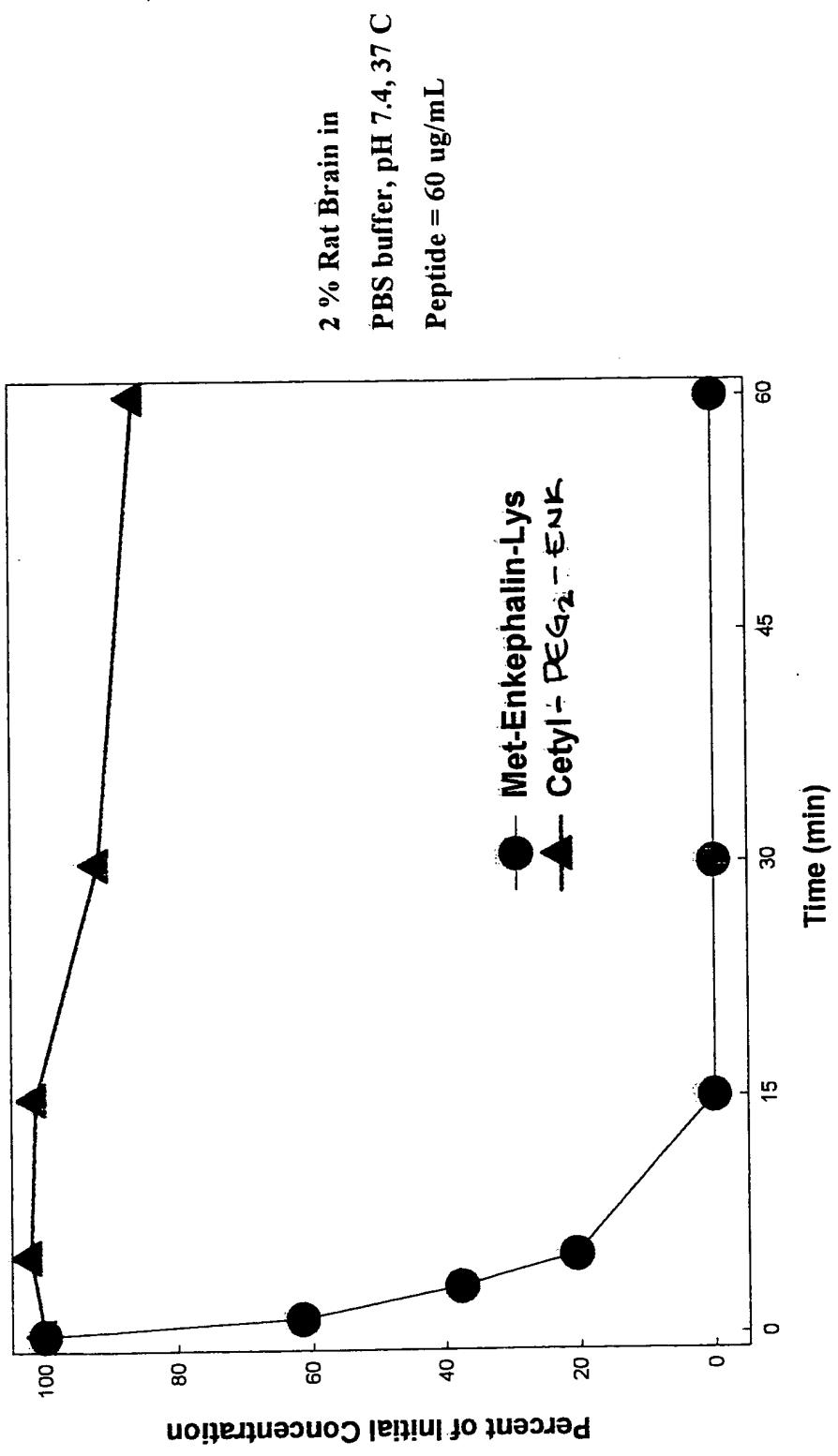
Cetyl- PEG$_2$ - ENK

FIGURE 2

Stability of Cetyl- PEG_2 Enkephalin

in Rat Brain Homogenate

2 % w/v Rat brain in

PBS buffer, pH 7.4, 37 C

Peptide = 60 ug/mL

Cetyl-TEG-Enkephalin Stability in Brain Homogenate
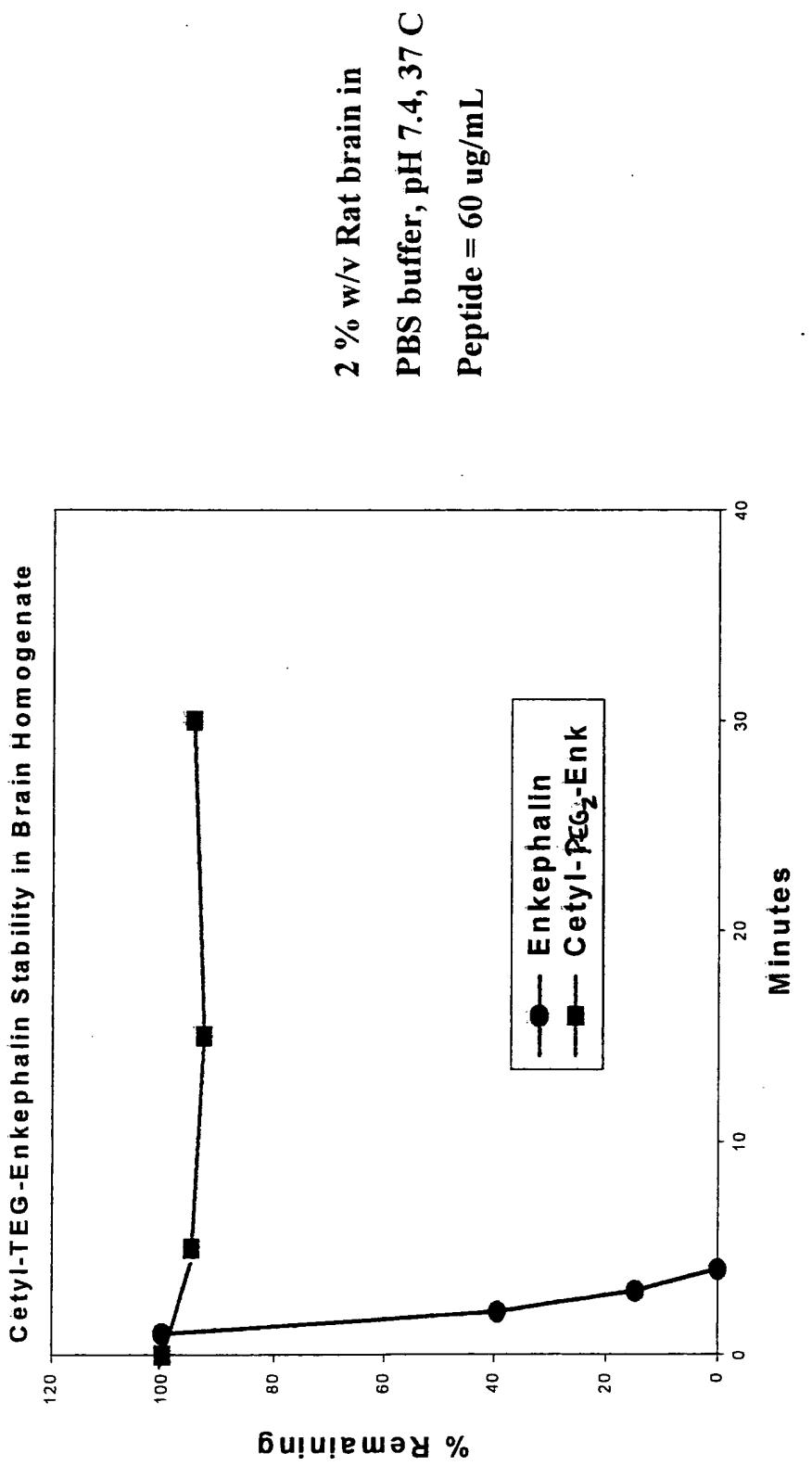
FIGURE 3

Stability of Palmitate-PEG₃-Enkephalin in Rat Brain Homogenate

2% w/v Rat Brain in

PBS buffer, pH 7.4, 37 C

Peptide = 60ug/mL

Stability of Palmitate-TEG-Enk in Brain homogenate
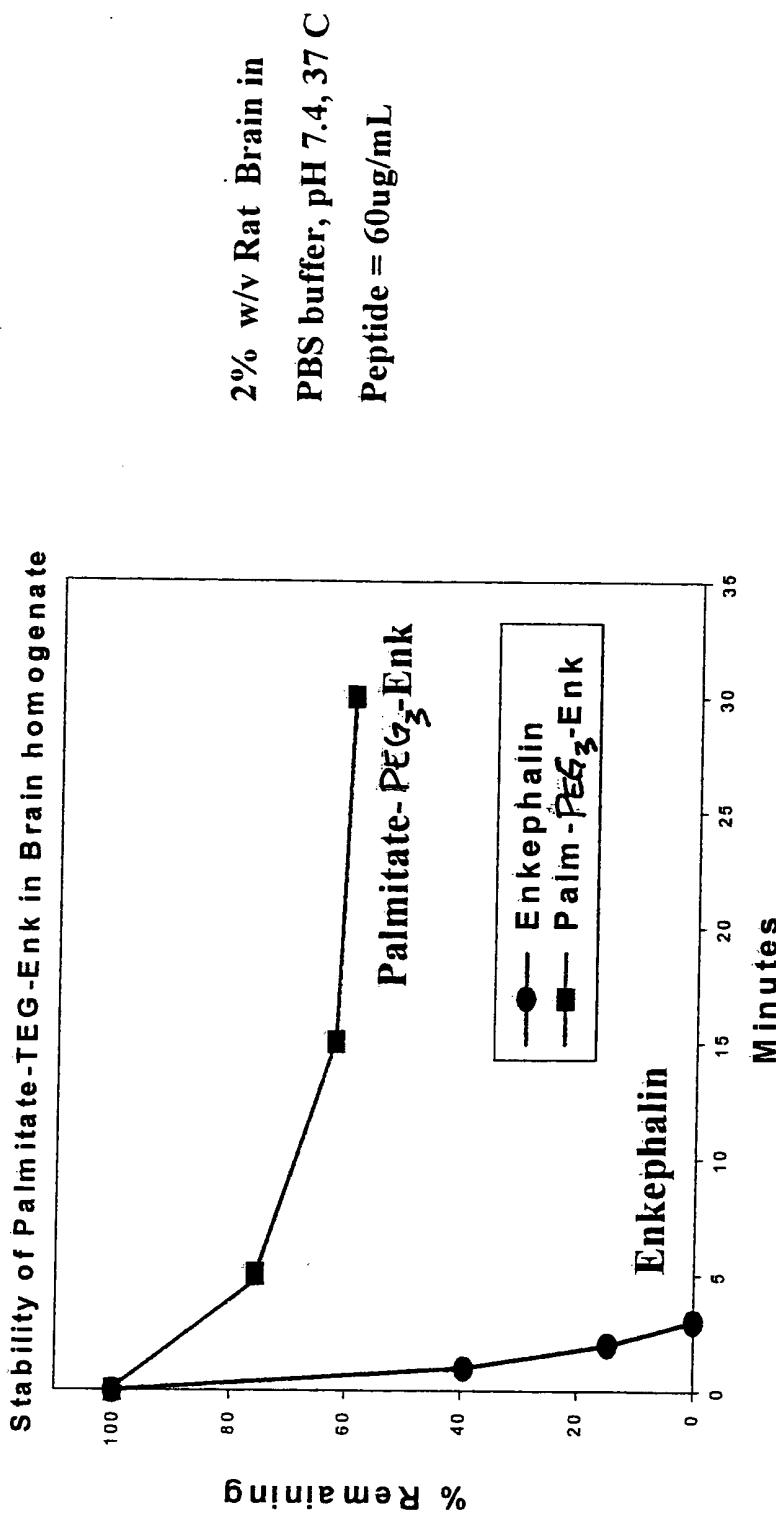
FIGURE 4

Isolation of Cetyl-PEG$_2$-Enkephalin from the Brain
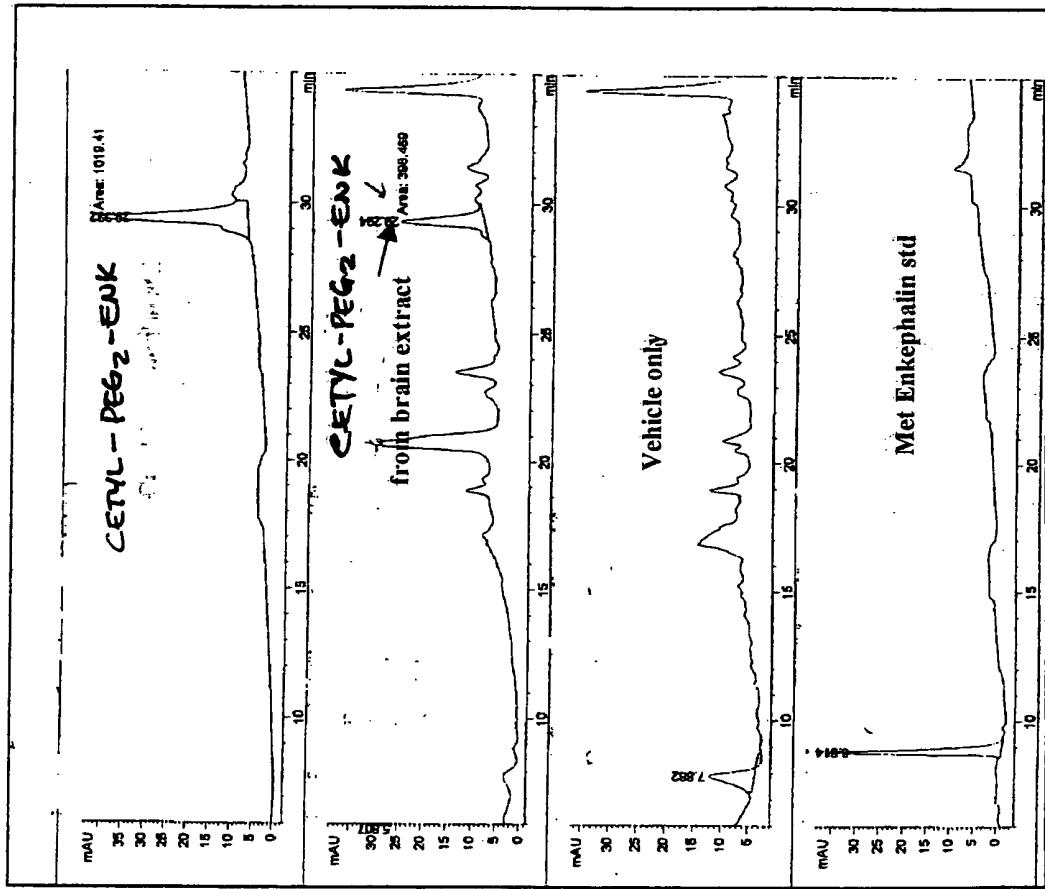
HPLC conditions:
Column: C-18
Solvent: solvent A: IPA
solvent B: Water
+0.1% TFA
Gradient: linear

CETYL-PEG$_2$-ENK

Area: 1019.41

FIGURE 5A

CETYL-PEG$_2$-ENK

from brain extract

Area: 398.469

FIGURE 5B

Vehicle only

FIGURE 5C

Met Enkephalin std

FIGURE 5D

## Naloxone Antagonism of Cetyl-PEG$_2$-Enkephalin-Induced Analgesia
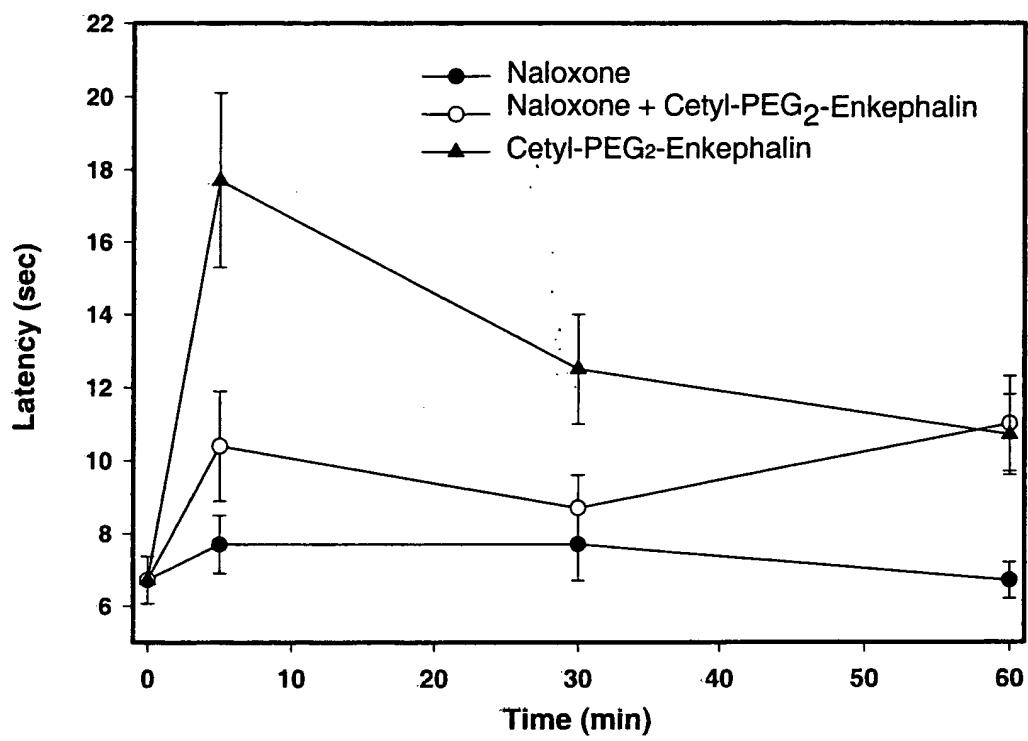
FIGURE 6

# Analgesic Effect of a 5 mg/kg IV Dose of Cetyl-PEG$_2$-Enkephalin Monoconjugate in the Rat Hot-Plate Assay
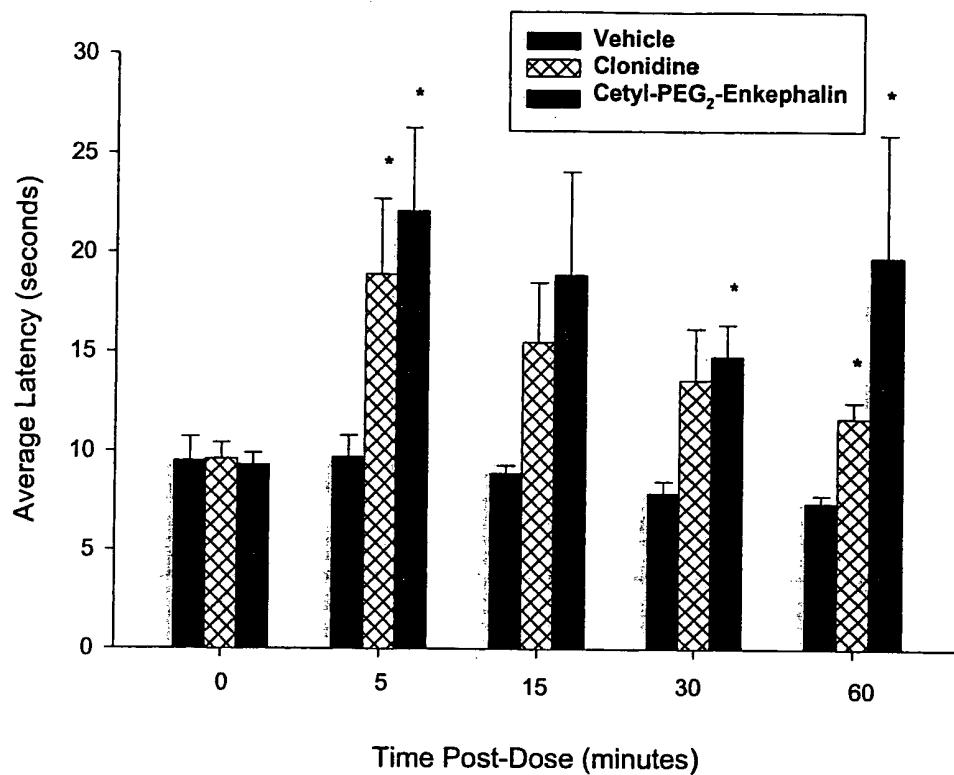
FIGURE 7

COMPARISON OF μ-RECEPTOR BINDING AFFINITY OF ENKEPHALIN CONJUGATES

DRUG OR CONJUGATE	DETAILED STRUCTURE	% SPECIFIC BINDING*
Naloxone	Naloxone	100
Enkephalin	Met-Enkephalin	67
Cetyl-ENK	Cetyl-PEG$_2$-ENK	100
Chol-ENK	Cholesterol-PEG$_3$-ENK	95
DHA-ENK	DHA-PEG$_2$-ENK	63
Palm-ENK	Palmitate-PEG$_3$-ENK	76
Cetyl-TEG-ENK	Cetyl-PEG$_3$-ENK	100

*Data are based on percent inhibition at a concentration of 100nM. The radioligand was DAMGO ([D-Ala2,N-Me-Phe-Gly5-ol]enkephalin) and naloxone served as the reference.

FIGURE 8

Synthesis of Oligomer

Polyethylene glycol

$m = 0, 1, 2, 3$
(average)

Base →

Addition →

Me-(CH₂)ₙ Br
(Bromo alkyl derivative)
m = 15, 17 etc.

Me

Amphiphilic Polymer

Me

Amphiphilic Polymer

"Free End"
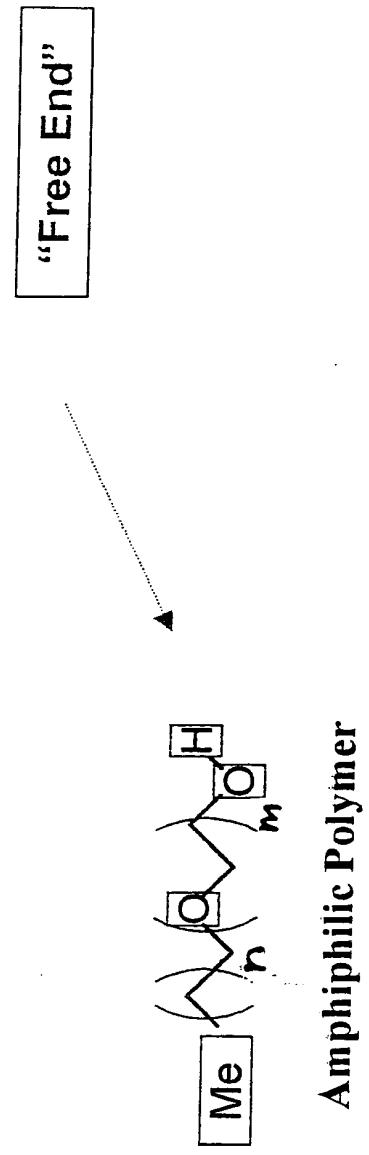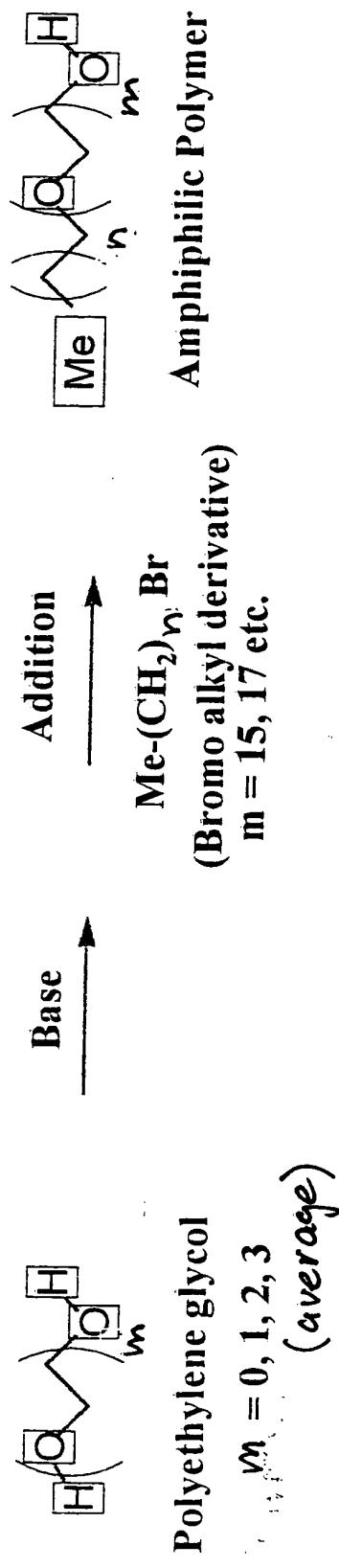
FIGURE 9

Attachment of Oligomer to Enkephalin

Me ⟶ "Free End"

Activation

Enkephalin

Oligomer-Enkephalin Conjugate
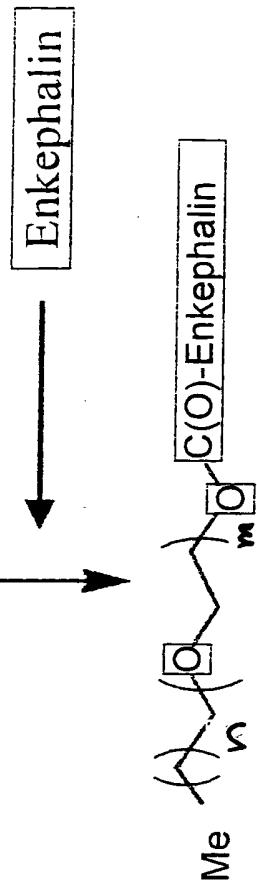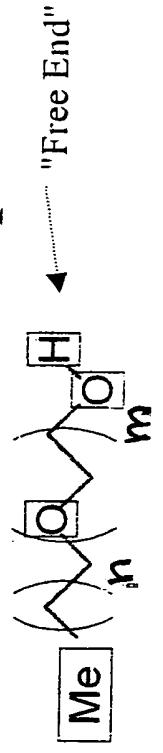
Me ⟶ C(O)-Enkephalin

Example | m = 14 and n = 2 | Cetyl-PEG₂-Enkephalin

FIGURE 10